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(71) Applicant *(for all designated States except AT, US)*: NOVARTIS AG [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH).

(71) Applicant *(for AT only)*: NOVARTIS PHARMA GMBH [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: PRUSCHY, Martin [CH/CH]; Bächlerweg 15, CH-8802 Kilchberg (CH).

(74) Agent: GRUBB, Philip; Novartis AG, Corporate Intellectual Property, CH-4002 Basel (CH).

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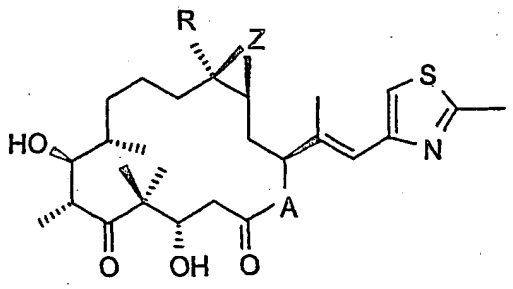
(54) Title: TREATMENT OF PROLIFERATIVE DISEASES WITH EPOTHILONE DERIVATIVES AND RADIATION



(I)

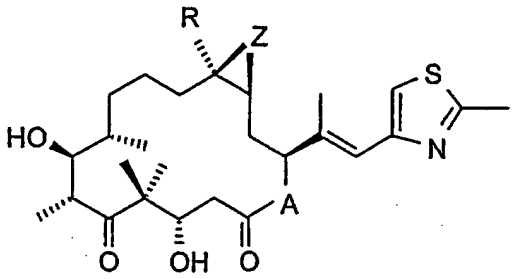
(57) Abstract: This invention relates to organic compounds of formula (I) in particular to pharmaceutical compositions for use in combination with ionizing radiation for the delay of progression or treatment of a proliferative disease, especially a solid tumor disease.

TREATMENT OF PROLIFERATIVE DISEASES WITH EPOTHILONE DERIVATIVES AND RADIATION

This invention relates to organic compounds, in particular to pharmaceutical compositions for use in combination with ionizing radiation for the delay of progression or treatment of a proliferative disease, especially a solid tumor disease.

We have now found that certain Epothilone derivatives are effective when used in combination with ionizing radiation for the delay of progression or treatment of a proliferative disease, especially a solid tumor disease;

Accordingly the invention provides a method for the delay of progression or treatment of a proliferative disease, especially a solid tumor disease in a subject in need of such treatment which comprises administering to the subject an effective amount of an epothilone derivative of formula I



(I)

in which compound A represents O or NRN, wherein RN is hydrogen or lower alkyl, R is hydrogen or lower alkyl, and Z is O or a bond, which is in free form or in the form of a pharmaceutically acceptable salt and optionally at least one pharmaceutically acceptable carrier; in combination with ionizing radiation.

A compound of formula I wherein A represents O, R is hydrogen and Z is O is known as epothilone A; a compound of formula I wherein A represents O, R is methyl and Z is O is known as epothilone B; a compound of formula I wherein A represents O, R is hydrogen and Z is a bond is known as epothilone C; a compound of formula I wherein A represents O, R is methyl and Z is a bond is known as epothilone D.

Further the invention provides the use of a compound of formula I (or pharmaceutically acceptable salt or prodrug ester thereof) for the preparation of a medicament for use in combination with ionizing radiation in the treatment of a proliferative disease.

In a further aspect the invention provides use of an epothilone derivative of formula I (or pharmaceutically acceptable salt or prodrug ester thereof) in combination with ionizing radiation for the treatment of a proliferative disease, especially a solid tumor.

In yet further aspect the invention provides an epothilone derivative of formula I (or pharmaceutically acceptable salt or prodrug ester thereof) as active ingredient for use in combination with ionizing radiation for the treatment of a proliferative disease, especially a solid tumor.

In still yet further aspect the invention provides a package comprising an epothilone derivative of formula I (or pharmaceutically acceptable salt or prodrug ester thereof) together with instructions for the use in combination with ionizing radiation for the treatment of a proliferative disease, especially a solid tumor.

Above and elsewhere in the present description the following terms have the meanings given below:

The term "lower" referred to above and hereinafter in connection with organic radicals or compounds respectively defines a compound or radical which may be branched or unbranched with up to and including 7, preferably up to and including 4 carbon atoms.

A lower alkyl group is branched or unbranched and contains 1 to 7 carbon atoms, preferably 1-4 carbon atoms. Lower alkyl represents, for example, methyl, ethyl, propyl, butyl, isopropyl or isobutyl.

The term "delay of progression" as used herein means administration of the combination to patients being in an early phase of the proliferative disease to be treated.

The term "solid tumor disease" as used herein comprises, but is not restricted to glioma, thyroid cancer, breast cancer, ovarian cancer, cancer of the colon and generally the GI tract, cervix cancer, lung cancer, in particular small-cell lung cancer, and non-small-cell lung cancer, head and neck cancer, bladder cancer, cancer of the prostate or Kaposi's sarcoma. In one preferred embodiment of the invention, the tumor disease to be treated is glioma, cancer of the prostate or thyroid cancer. The present combination inhibits the growth of solid

tumors, but also liquid tumors. Furthermore, depending on the tumor type and the particular combination used, a decrease of the tumor volume can be obtained. The combinations disclosed herein are also suited to prevent the metastatic spread of tumors and the growth or development of micrometastases.

Combination refers to administration of an amount of epothilone derivative of formula I in combination with administration of an amount of ionizing radiation such that there is a synergistic effect which would not be obtained if an epothilone derivative of formula I is administered without separate, simultaneous or sequential administration of ionizing radiation. Wherein administration of ionizing radiation can be continuous, sequential or sporadic. Or an effect which would not be obtained if there is administered ionizing radiation without the separate, simultaneous or sequential administration of an Epothilone derivative of formula I, wherein administration can be continuous, sequential or sporadic

Preferably combination refers to administration of an amount of epothilone derivative of formula I in combination with administration of an amount of ionizing radiation such that there is a synergistic antiproliferative effect and/ or a clonogenic cell killing effect that would not be obtained if

a) The epothilone derivative of formula I is administered without prior, simultaneous or subsequent administration of ionizing radiation. Wherein administration can be continuous, sequential or sporadic;

b) There is administration of ionizing radiation without the prior, simultaneous or subsequent administration of an epothilone derivative of formula I. Where in administration can be continuous, sequential or sporadic.

The term "ionising radiation" referred to above and hereinafter means ionising radiation that occurs as either electromagnetic rays (such as X-rays and gamma rays) or particles (such as alpha and beta particles). Ionising radiation is provided in, but not limited to, radiation therapy and is known in the art (Hellman, Principles of Radiation Therapy, Cancer, in Principles and Practice of Oncology, 248-275 (Devita et al., ed., 4th Ed., V1, 1993).

Epothilone derivatives of formula I wherein A represents O or NRN, wherein RN is hydrogen or lower alkyl, R is hydrogen or lower alkyl and Z is O or a bond, and methods for the preparation of such epothilone derivatives are in particular generically and specifically disclosed in the patents and patent applications WO 93/10121, US 6,194,181, WO

98/25929, WO 98/08849, WO 99/43653, WO 98/22461 and WO 00/31247 in each case in particular in the compound claims and the final products of the working examples, the subject-matter of the final products, the pharmaceutical preparations and the claims is hereby incorporated into the present application by reference to this publications. Comprised are likewise the corresponding stereoisomers as well as the corresponding crystal modifications, e.g. solvates and polymorphs, which are disclosed therein.

The transformation of epothilone B to the corresponding lactam is disclosed in Scheme 21 (page 31, 32) and Example 3 of WO 99/02514 (pages 48 - 50). The transformation of a compound of formula I which is different from epothilone B into the corresponding lactam can be accomplished analogously. Corresponding epothilone derivatives of formula I wherein RN is lower alkyl can be prepared by methods known in the art such as a reductive alkylation reaction starting from the epothilone derivative wherein RN is hydrogen.

Epothilone derivatives of formula I, especially epothilone B, can be administered as part of pharmaceutical compositions which are disclosed in WO 99/39694.

A combination which comprises (a) an epothilone derivative of formula I in which compound A represents O or NRN, wherein RN is hydrogen or lower alkyl, R is hydrogen or lower alkyl, and Z is O or a bond, which may be present in free form or in the form of a pharmaceutically acceptable salt and optionally at least one pharmaceutically acceptable carrier and (b) ionizing radiation, will be referred to hereinafter as a COMBINATION OF THE INVENTION.

The nature of proliferative diseases like solid tumor diseases is multifactorial. Under certain circumstances, drugs with different mechanisms of action may be combined. However, just considering any combination of drugs having different mode of action does not necessarily lead to combinations with advantageous effects.

In the combination of the invention, Epothilone derivatives of formula I and pharmaceutically acceptable salts and prodrug derivatives are preferably used in the form of pharmaceutical preparations that contain the relevant therapeutically effective amount of active ingredient optionally together with or in admixture with inorganic or organic, solid or liquid, pharmaceutically acceptable carriers which are suitable for administration.

In a preferred embodiment, each patient receives doses of ionizing radiation, whereas the epothilone derivative of formula I is administered once weekly i.v. for three weeks, followed by one week off. Each four week interval will be considered one cycle. Day 1 of each cycle is

defined as the day of administration of epothilone derivative of formula I and ionizing radiation. The efficacy of the treatment can be determined in these studies, e.g., after 18 or 24 weeks by radiologic evaluation of the tumors every 6 weeks.

In an alternative embodiment, the ionizing radiation is given as a pre-treatment, i.e. before the treatment with the COMBINATION OF THE INVENTION is started; the ionizing radiation alone is administered to the patient for a defined period of time, e.g. daily administration of the ionizing radiation alone for two or three days or weeks.

In another preferred embodiment, the epothilone derivative of formula I is administered once weekly i.v. for three weeks, followed by one week off. Each four week interval will be considered one cycle. The efficacy of the treatment can be determined in these studies, e.g., after 18 or 24 weeks by radiologic evaluation of the tumors every 6 weeks.

The Epothilone derivative pharmaceutical compositions may be, for example, compositions for enteral, such as oral, rectal, aerosol inhalation or nasal administration, compositions for parenteral, such as intravenous or subcutaneous administration, or compositions for transdermal administration (e.g. passive or iontophoretic), or compositions for topical administration,

Preferably, the Epothilone derivative pharmaceutical compositions are adapted to oral administration.

The pharmaceutical compositions according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including man, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone or in combination with one or more pharmaceutically acceptable carries, especially suitable for enteral or parenteral application.

The novel pharmaceutical composition contain, for example, from about 10 % to about 100 %, preferably from about 20 % to about 60 %, of the active ingredients. Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, and furthermore ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-

coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached by administration of a plurality of dosage units.

In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavouring agents, preservatives, colouring agents; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, capsules and tablets, with the solid oral preparations being preferred over the liquid preparations. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed.

In particular, a therapeutically effective amount of each combination partner of the COMBINATION OF THE INVENTION may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of delay of progression or treatment of a proliferative disease according to the invention may comprise (i) administration of the first combination partner and (ii) administration of the second combination partner, wherein administration of a combination partner may be simultaneous or sequential in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or weekly dosages corresponding to the amounts described herein. The individual combination partners of the COMBINATION OF THE INVENTION can be administered separately at different times during the course of therapy or concurrently. Furthermore, the term administering also encompasses the use of a pro-drug of an epothilone derivative of formula I that converts *in vivo* to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The dosage of ionizing radiation and an epothilone derivative of formula I in relation to each other is preferably in a ratio that is synergistic.

If the warm-blooded animal is a human, the dosage of a compound of formula I is preferably in the range of about 0.25 to 75, preferably 0.5 to 50, e.g. 2.5, mg/m^2 once weekly for two to four, e.g. three, weeks, followed by 6 to 8 days off in the case of an adult patient. In one

embodiment of the invention, epothilone B is administered in accordance with the treatment schedule described in US 6,302,838 which disclosure is enclosed herein by reference.

The particular mode of administration and the dosage of a compound of formula I may be selected by the attending physician taking into account the particulars of the patient, especially age, weight, life style, activity level, etc.

The dosage of an epothilone derivative of formula I may depend on various factors, such as effectiveness and duration of action of the active ingredient, mode of administration, effectiveness and duration of action of the ionizing radiation and/or sex, age, weight and individual condition of the subject to be treated.

The dosage of ionizing radiation may depend on various factors, such as effectiveness and duration of action of the ionizing radiation, mode of administration, location of administration, effectiveness and duration of action of the epothilone derivative of formula I and/or sex, age, weight and individual condition of the subject to be treated. The dosage of ionizing radiation is generally defined in terms of radiation absorbed dose, time and fraction, and must be carefully defined by the attending physician.

Salt-forming groups in a compound of formula I are groups or radicals having basic or acidic properties. Compounds having at least one basic group or at least one basic radical, for example a free amino group, a pyrazinyl radical or a pyridyl radical, may form acid addition salts, for example with inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxy-benzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic sulfonic acids, for example benzene-, p-toluene- or naphthalene-2-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed.

For the purposes of isolation or purification, as well as in the case of compounds that are used further as intermediates, it is also possible to use pharmaceutically unacceptable salts.

Only pharmaceutically acceptable, non-toxic salts are used for therapeutic purposes, however, and those salts are therefore preferred.

In the compound of formula I preferably A represents O. R is lower alkyl, e.g. ethyl or, most preferably, methyl. Z is preferably O.

In one preferred embodiment of the invention the combination comprises epothilone B and ionizing radiation.

Moreover, the present invention relates to a method of treating a warm-blooded animal having a proliferative disease comprising administering to the animal a COMBINATION OF THE INVENTION in a way that is jointly therapeutically effective against a proliferative disease and in which the combination partners can also be present in the form of their pharmaceutically acceptable salts.

Furthermore, the present invention pertains to the use of a COMBINATION OF THE INVENTION for the delay of progression or treatment of a proliferative disease and for the preparation of a medicament for the delay of progression or treatment of a proliferative disease.

In one embodiment of the invention, an antidiarrheal agent is administered together with the COMBINATION OF THE INVENTION in order to prevent, control or eliminate diarrhoea that is sometimes associated with the administration of epothilones, especially epothilone B. Thus, the present invention also relates to a method of preventing or controlling diarrhoea associated with administering an epothilone derivative of formula I, which comprises administering an effective amount of an antidiarrhea agent to the patient receiving treatment with the COMBINATION OF THE INVENTION. Antidiarrheal agents and protocols for their administration are known to those skilled in the art. Antidiarrheal agents suitable for use in the inventive methods and compositions include, but are not limited to, natural opiods, such as tincture of opium, paregoric, and codeine, synthetic opioids, such as diphenoxylate, difenoxin and loperamide, bismuth subsalicylate, octreotide (e.g. available as SANDO-STATINTM), motilin antagonists and traditional antidiarrheal remedies, such as kaolin, pectin, berberine and muscarinic agents.

The following example is intended to illustrate the invention and are not to be construed as being limitations thereon.

Example 1

Tumor cell proliferation was assessed by the colorimetric MTT-like alamarBlue assay that is based on detection of metabolite activity. To determine clonogenic survival the number of singular cells plated was adjusted to obtain about 100 colonies per dish with a given treatment. After 24 h exposure to the different drugs cells were irradiated and then allowed to grow for 8 to 10 days before fixation in methanol/acetic acid (75%/25%) and staining with crystal violet. Only colonies with more than 50 cells/colony were counted. The plating efficiency (PE) of untreated cells was determined and calculated as PE (%) = (scored colonies/number of plated cells)x100. The surviving fraction (SF) with a given treatment was determined by SF = (scored colonies) / (number of plated cells x PE/100). Clonogenic assays were performed at least twice and absence of error bars is due to minimal standard deviations. Irradiation of cell cultures was carried out at RT in tissue culture dishes (100x100mm) or in 96-well plates using a Pantak Therapax 3,300 kV X-ray unit at 0.7 Gy/min. Dosimetry was controlled with a Vigilant-dosimeter.

Initial proliferation assays were performed to determine the dose range of epothilone B to be applied for combined treatment with ionizing radiation. A clear antiproliferative dose response over 72 hours against the two cell lines (E1A/ras transformed p53-/- MEF; human colon adenocarcinoma cell line SW480) was observed in a subnanomolar and low nanomolar range of epothilone B. The human SW480 cells showed enhanced sensitivity to epothilone B than the genetically defined oncogene-transformed MEFs (Appendix epothilone B-1). Combined treatment with epothilone B and ionizing radiation (5Gy) revealed an at least additive antiproliferative effect against these two cell lines (Appendix epothilone B-2, shown with a representative concentration of epothilone B). For this combined treatment modality cells were pretreated for 24h with epothilone B prior to irradiation.

Based on these results, clonogenic survival assays were performed with epothilone B in combination with ionizing radiation. The clonogenic assay is based on the outgrowth of compact clones from singular cells that are seeded at low density in a petri dish. Clonal outgrowth under the different treatment conditions can be quantified and compared.

Appendix epothilone B-3 summarizes clonogenic survival performed with SW480 and E1A/ras-trasnsormed MEFs upon treatment with ionizing radiation and epothilone B alone and in combination. Similar to the proliferation assay SW480 cells were more sensitive to epothilone B than the MEFs and combined treatment showed again an at least additive effect in both cell lines (dose range for epothilone B are different for SW480 and MEFs as

illustrated in the respective graphs). Based on these results efficacy of combined treatment should be tested in vivo using tumor allograft/xenograft models.

Based on the interesting profile of epothilone B to be antiproliferative also in (some) Paclitaxel-refractory tumor cell lines we compared the effect of epothilone B and Paclitaxel in combination with ionizing radiation against these two cell lines. The human SW480 colon cancer cell line proofed to be refractory to Paclitaxel (doses up to 500 nM, Appendix 4a).

Though Paclitaxel and epothilone B reduced the proliferative activity alone and in combination with ionizing radiation in the murine fibrosarcoma cell line to a comparable extent (in a low nanomolar range, Appendix 4b).

We compared the effect of combined treatment with Epothilone B/IR and Paclitaxel/IR also in the clonogenic cell survival assay. Both cell lines showed an at least additive effect to combined treatment with epothilone B/IR and Paclitaxel /IR respectively, but only at very high concentrations of Paclitaxel in the Paclitaxel-resistant cell line SW480 (Appendix epothilone B-5).

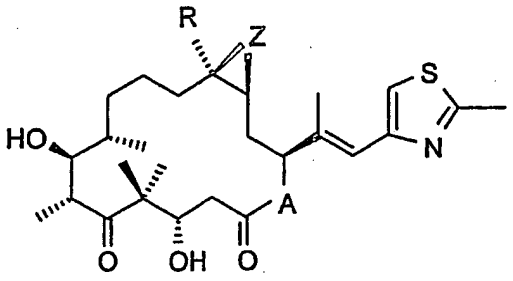
Paclitaxel-resistance is often due to MDR-P-glycoprotein-overexpression in tumor cells, inhibitable by the MDR-reversal agent verapamil. Therefore proliferation experiments were performed with the different treatment modalities in SW480 cells pretreated with verapamil. Low doses of verapamil (5µg/ml, added 30 min prior to Paclitaxel-treatment) resensitized SW480 cells to low doses of Paclitaxel alone and to a combined treatment modality with ionizing radiation indicating that MDR-P-glycoprotein-overexpression is responsible for the Paclitaxel-refractory effect in this cell line (Appendix epothilone B-6). Verapamil did not have an antiproliferative effect by itself at this concentration and only slightly affected the response to epothilone B (not shown). We are currently probing the level of MDR-P-glycoprotein in a direct way in SW480 cells by immunoblotting.

Overall these results show that both epothilone B and paclitaxel have an at least additive antiproliferative and clonogenic cell killing effect in combination with ionizing radiation. epothilone B retains full activity alone and in combination with ionizing radiation in the Paclitaxel-resistant human colon adenocarcinoma cell line SW480. Thus Epothilone might be a promising alternative in paclitaxel resistant tumors (e.g. colorectal tumors) for a combined treatment regimen using IR and microtubule inhibitors. Experimentally the next steps will involve in vivo testing of epothilone B/IR with allograft/xenograft mouse tumor models.

Claims

1. A method for treating a proliferative disease in a subject in need of such treatment, wherein the method comprises administering;

(a) an epothilone derivative of formula I



(I)

in which compound A represents O or NRN, wherein RN is hydrogen or lower alkyl, R is hydrogen or lower alkyl, and Z is O or a bond, which is in free form or in the form of a pharmaceutically acceptable salt, in combination with

(b) ionizing radiation.

2. A method according to claim 1 which comprises administering

(a) a compound of formula I wherein A represents O. R is lower alkyl, Z is O, in combination with

(b) ionizing radiation.

3. A method according to claim 1 or 2 which comprises administering

(a) epothilone B, in combination with

(b) ionizing radiation.

4. A method according to claims 1, 2 or 3 wherein subject is a warm-blooded animal having a proliferative disease comprising administering to the animal a combination according to any of claims 1, 2 or 3 in a way that is jointly therapeutically effective against a proliferative disease.

5. A method according to any of claims 1 to 4 which comprises administering a quantity which is jointly therapeutically effective against a proliferative disease of a compound of formula I and at least one pharmaceutically acceptable carrier for use in combination with ionizing radiation.

6. A method according to claim 1, 2, 3, 4 or 5 for the delay of progression of a proliferative disease in a subject in need of such treatment.

7. A method according to claim 1, 2, 3, 4 or 5 for the treatment of a proliferative disease.

8. Use of a compound of formula I according to claim 1, 2 or 3 for the preparation of a medicament for use in combination with ionizing radiation for the delay of progression or treatment of a proliferative disease.

10. A method according to claims 6, 7 or 8 wherein the proliferative disease is a solid tumor.

11. A package comprising a compound of formula I in which A represents O or NRN, wherein RN is hydrogen or lower alkyl, R is hydrogen or lower alkyl, and Z is O or a bond (or pharmaceutically acceptable salt or prodrug ester thereof), together with instructions for the use in combination with ionizing radiation for the treatment of a proliferative disease.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/002610

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/427 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/02514 A (SQUIBB BRISTOL MYERS CO) 21 January 1999 (1999-01-21) cited in the application page 1 - page 3 page 8, line 20 - page 9, line 11 page 10, paragraph 2; example 1 -----	1-11
X	WO 02/058700 A (SQUIBB BRISTOL MYERS CO) 1 August 2002 (2002-08-01) page 1 - page 3 page 9, line 8 - page 10, line 12 page 11, lines 11,12 example 2 -----	1-11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2004	13. 07. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Paul Soto, R

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 2002, no. 02, 2 April 2002 (2002-04-02) & JP 2001 288097 A (PG-TXL CO LP), 16 October 2001 (2001-10-16) abstract -----	1-11
X,P	KIM JAE-CHUL ET AL: "Potential radiation-sensitizing effect of semisynthetic epothilone B in human lung cancer cells." RADIOTHERAPY & ONCOLOGY, vol. 68, no. 3, September 2003 (2003-09), pages 305-313, XP002283036 ISSN: 0167-8140 the whole document -----	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/002610

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-7 and 10 (industrial applicability)
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-7 and 10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

[redacted]formation on patent family members

Inter[redacted]tal Application No
PCT/EP2004/002610

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9902514 A	21-01-1999	AU 731497 B2	29-03-2001
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JP 2001288097 A	16-10-2001	NONE	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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(10) International Publication Number
WO 2004/007476 A1

(51) International Patent Classification[7]: C07D 313/00, 327/02, 417/06, 497/04, 493/04, A61K 31/425, A61P 35/00

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(22) International Filing Date: 10 June 2003 (10.06.2003)

(25) Filing Language: English

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(30) Priority Data:
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(71) Applicant *(for all designated States except US)*: **GESELLSCHAFT FUER BIOTECHNOLOGISCHE FORSCHUNG MBH (GBF)** [DE/DE]; Mascheroder Weg 1, 38124 Braunschweig (DE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **HOEFLE, Gerhard** [DE/DE]; Alter Weg 12a, 38124 Braunschweig (DE).

(74) Agents: **BOETERS, Hans** et al.; Boeters & Lieck, Bereiteranger 15, 81541 München (DE).

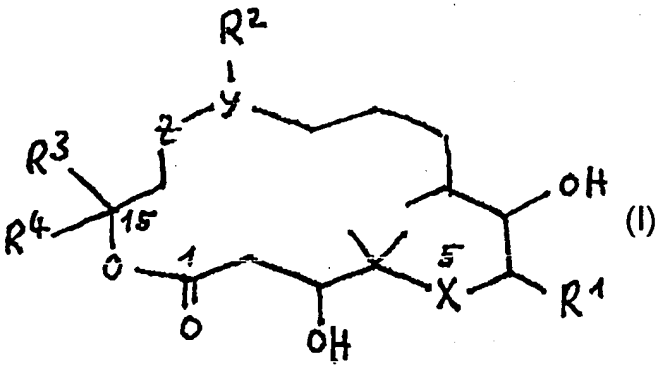
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG MBH (GBF)



(I)

(57) Abstract: The invention relates to 5-thiapethilones and 15-disubstituted epothilones according to formula I (I) with the following meanings: X= >C = O or >S = O R^1 = C_{1-6} alkyl or C_{2-6} alkenyl R^2 = H or C_{1-6} alkyl Y - Z = >C=C< or >C-O-C< (epoxide ring) R^3 = H, C_{1-6} alkyl or C_{2-6} alkenyl R^4= bicycloaryl, bicycloheteroaryl or -C(R^5) = CH-R^6, where R^5 = H or CH_3 and R^6 = aryl or heteroaryl X not being >C=O if R^3 = H.

WO 2004/007476 A1

Gesellschaft für Biotechnologische Forschung mbH (GBF)

5-THIAEPOTHILONES AND 15-DISUBSTITUTED EPOTHILONES

The present invention relates to 5-thiaepothilones and 15-disubstituted epothilones which are 16-membered cytotoxic macrolides of formula I with an application potential in cancer therapy and in the treatment of other instances of cell growth impairment.

Epothilones are well known. They can be obtained by fermenting the myxobacterium Sorangium cellulosum (GBF) by semisynthesis (GBF, BMS) by genetic engineering and heterologous expression (Kosan Biosciences), by total synthesis (Danishefsky, Nicolaou, Schinzer, Novartis, Schering).

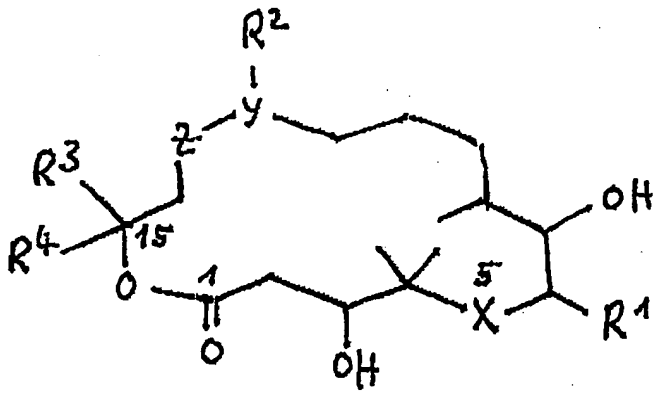
All the epothilones which have become known so far have the common characteristic of carrying a keto group (X = carbonyl) in position 5 and a hydrogen (R^3 = H) on the C15 atom. The present invention relates to epothilones which, in contrast to the known state of the art, exhibit either

(1) a sulphoxide group for X or

(2) an alkyl or alkenyl group by way of R^3 on the C15 carbon atom or

(3) both a sulphoxide group X and an alkyl or alkenyl group as radical R^3.

The invention also relates to epothilones of the following general formula I:



I

with the following meanings:

X = >C = O or >S = O
R^1 = C_{1-6} alkyl or C_{2-6} alkenyl
R^2 = H or C_{1-6} alkyl
Y - Z = >C=C< or >C-O-C< (epoxide ring)
R^3 = H, C_{1-6} alkyl or C_{2-6} alkenyl
R^4 = bicycloaryl, bicycloheteroaryl or $-C(R^5) = CH-R^6$,

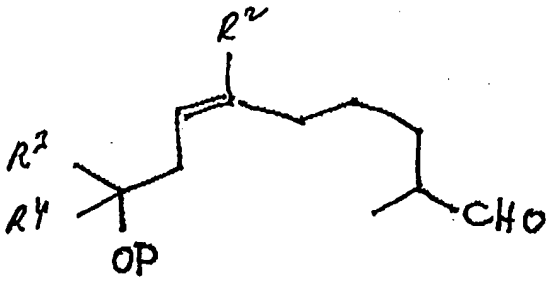
where

R^5 = H or CH_3 and
R^6 = aryl or heteroaryl
X not being >C=O if R^3 = H.

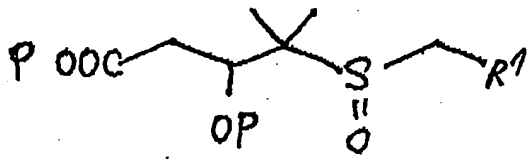
A compound of the general formula I with Z-Y = >C=C< can be produced from a compound of formula 1 by aldol reaction with a compound of formula 2. In the following reaction scheme, P represents a protective group common in epothilone chemistry, such as a silyl group. Subsequently, the compound of formula 3 thus obtained is reacted, with ring closure (formation of lactone), to a compound of formula 4.

A compound of the general formula I with Y-Z = >C-O-C< (epoxide ring) can be produced by reacting a compound of formula 5 with a compound of formula 6 in an aldol

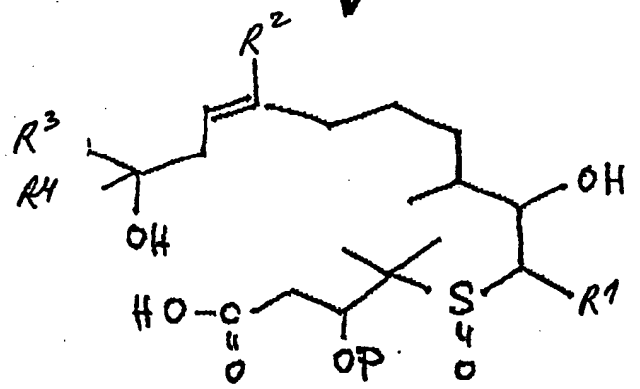
reaction. The resulting compound of formula 7 can be cyclised after liberating the aldehyde group from the acetal in an aldol reaction, whereupon the lactone thus obtained is subjected to epoxidation in position 12,13.


R2
R3
R4
OP
CHO

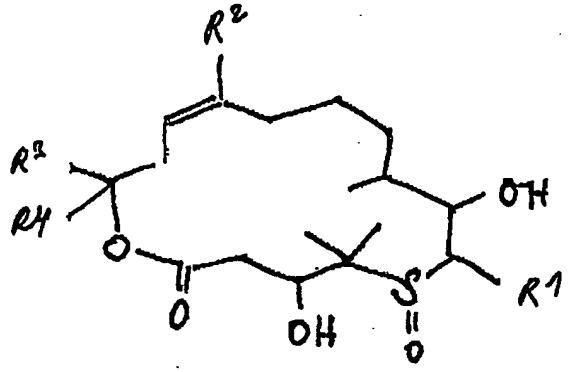
1


P OOC
OP
S
O
R1

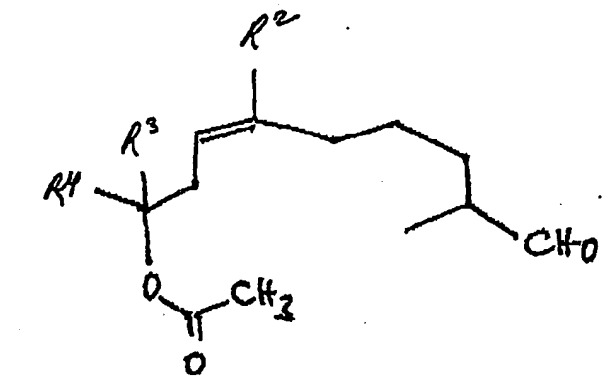
2


R2
R3
R4
OH
OH
HO-C
O
OP
S
O
R1

3


R2
R3
R4
O
O
OH
OH
S
O
R1

4



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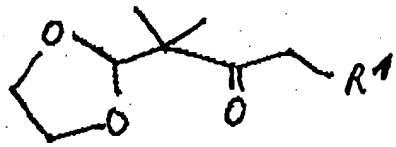

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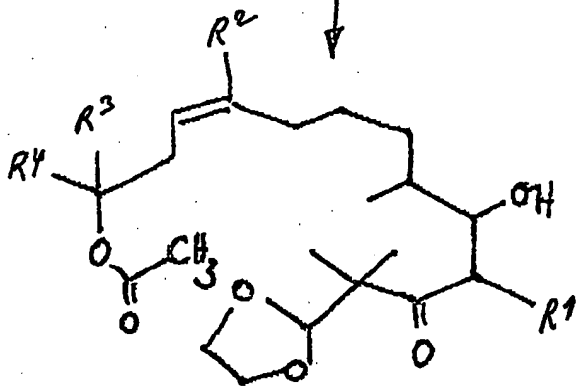

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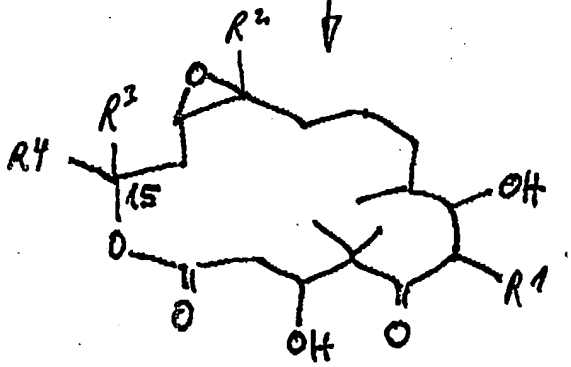
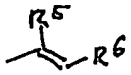


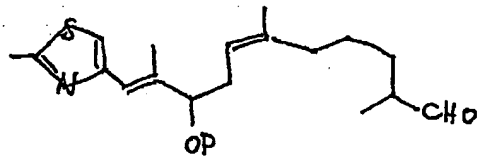
8

Below, the invention is further illustrated by two synthesis examples.

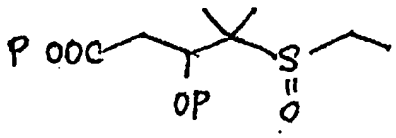
Synthesis example Ia: X = SO, R^1, R^2 = CH_3,

Z - Y = C=C, R^3 = H, R^4 = 

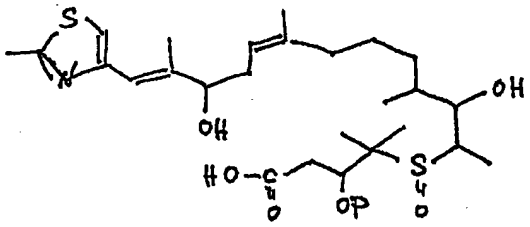
with R^5 = CH_3 , R^6 = 4-(2-methylthiazolyl)

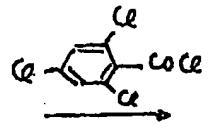


$(Me_3Si)_2NLi$ →



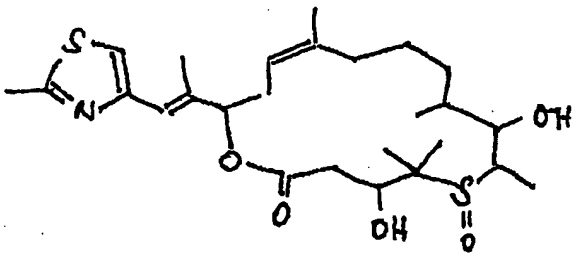
P = protective groups, e.g. silyl





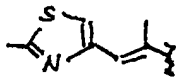
HF →

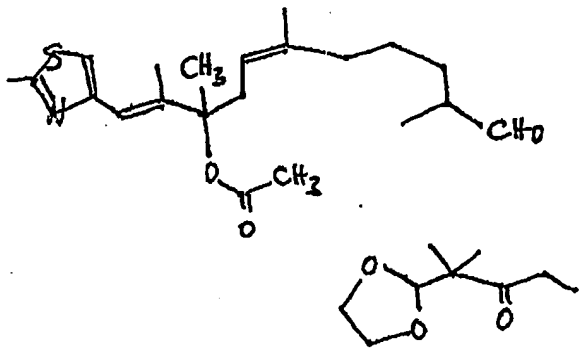
Net_3, DMAP, pyiridine



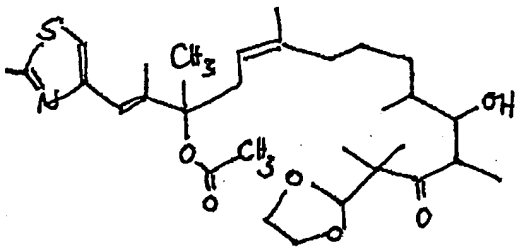
5-thiaepothilone

Synthesis example Ib: X = C = O, R^1, R^2 = CH_3,

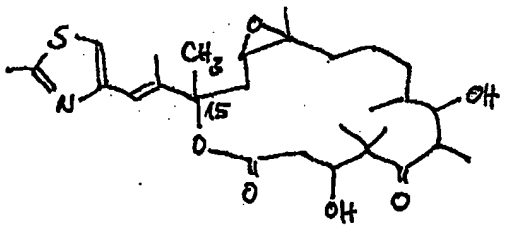
Z - Y =  R^3 = CO_3, R^4 = 



LDA



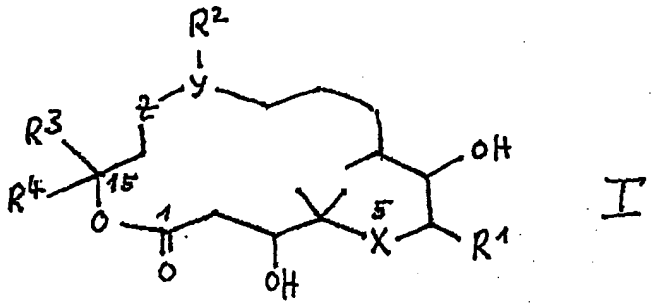
1. Cerium-ammonium nitrate
2. + BnLi
3. Dimethyl dioxirane



= 15-Methyl epothilone B

CLAIMS

1. Epothilone of the general formula (I):



I

with the following meanings:

X = >C = O or >S = O and/or
R^1 = C_{1-6} alkyl or C_{2-6} alkenyl and/or
R^2 = H or C_{1-6} alkyl and/or
Y - Z = >C=C< or >C-O-C< (epoxide ring) and/or
R^3 = H, C_{1-6} alkyl or C_{2-6} alkenyl and/or
R^4 = bicycloaryl, bicycloheteroaryl or $-C(R^5) = CH-R^6$,

where

R^5 = H or CH_3 and
R^6 = aryl or heteroaryl,
X not being >C=O if R^3 = H,

and one, a plurality or all conceivable combinations of the radicals X, R^1, R^2, R^3, R^4, R^5, R^6 and Y - Z

2. Epothilone according to claim 1, where R^4 is a bicycloaryl or bicycloheteroaryl radical common in epothilone chemistry.

3. Epothilone according to claim 1, where R^6 is an aryl or heteroaryl radical common in epothilone chemistry.

4. Epothilone according to claim 3, where the heteroaryl radical is a monocyclic 5 or 6-membered heteroaromatic which may exhibit one or a plurality of O and/or N and/or S atoms in the ring.

5. Epothilone according to claim 3, where the aryl radical may be a heteroaromatic with one or a plurality of and in particular 1, 2, 3 or 4 heteroatoms.

6. Agent for cancer therapy and/or treating other instances of cell growth impairment, consisting of or containing one or a plurality of epothilones according to any one of the preceding claims, apart from the usual auxiliary agents.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/06066

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D313/00 C07D327/02 C07D417/06 C07D497/04 C07D493/04
A61K31/425 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 02514 A (BRISTOL-MYERS) 21 January 1999 (1999-01-21) page 64; claims 1,3-5	1,3,6

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 October 2003	14/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Francois, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/06066

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		TR 200000065 T2	21-11-2000
		US 6605599 B1	12-08-2003
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		ZA 9805938 A	10-01-2000